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POLYPEPTIDE WITH 46 KDALTON HMFG DIFFERENTIATION ANTIGEN BINDING SPECIFICITY AND CLOTTING FACTORS V AND VIII LIGHT-CHAIN HOMOLOGIES, FUSION PROTEIN, POLYNUCLEOTIDE AND POLYRIBONUCLEOTIDE ENCODING THE POLYPEPTIDE, ANTI-POLYPEPTIDE ANTIBODIES, KITS AND METHODS OF USE THEREOF

Technical Field

This invention relates to a polypeptide having the antibody binding specificity of the 46 kDalton HMFG differentiation antigen, a polynucleotide, and a polyribonucleotide encoding it, anti-polypeptide antibodies, methods of detecting the polypeptide and DNA and RNA encoding it, a method of imaging cells expressing the polypeptide, a method of detecting the presence of the polypeptide in a biological fluid by binding the antibody to the polypeptide, in vivo and ex vivo methods of delivering a therapeutic agent to a target cell expressing the polypeptide, a fusion protein of the polypeptide and at least one other polypeptide, labeled polynucleotides and polyribonucleotides encoding the polypeptide and a complementary DNA sequence, method of detecting RNA and DNA by hybridization with labeled probes, a method of vaccination with the polypeptide, and method of treating breast cancer with an anti-sense DNA.

Background Art

The human milk fat globule (HMFG) has been used extensively as a source of antigenic material for the preparation of both polyclonal and monoclonal antibodies that have found widespread use in the diagnosis of breast cancer, as well as in the study of the breast epithelial cell surface and the processing of its antigenic components.

Polyclonal antiserum was originally prepared, that after appropriate absorptions with non-breast tissue was found to identify surface antigens of human mammary epithelial cells (HME-Ags). This antiserum (anti-HME) had a high specificity for normal breast epithelial cells and breast carcinomas. It identified mainly three components of the human milk fat globule which had molecular weights of 150 kDa, 70 kDa, and 46 kDa, respectively.

Monoclonal antibodies were first made against the HMFG in 1980. These antibodies were applied to identify a hitherto unknown component of the breast epithelial cell surface, a large molecular weight mucin-like glycoprotein, that was named non-penetrating glycoprotein (NPGP). This latter component appears to be extremely antigenic in the mouse. The vast majority of monoclonal antibodies prepared against HMFG as well as breast tumors have been found to have specificity against different epitopes of this mucin complex. Less frequently, monoclonal antibodies have been prepared against the 70 kDa and 46 kDa components of the HMFG.

The reason for the high immunogenicity of NPGP has recently been elucidated by the characterization of cDNA clones selected from a kgtll breast cell library using both polyclonal and monoclonal antibodies against the mucin. These cDNA clones consist of large arrays of

highly conserved 60 bp tandem repeats. The resulting 20 amino acid repeat contains epitopes for several anti-mucin antibodies.

The repeat is apparently unstable at the genomic level. This may account for the observed polymorphism seen at the gene, RNA and protein levels for this high molecular weight mucin. An initial report on cDNA cloning of the mucin product suggested that the core protein had a molecular weight of about 68 kDa. However, the mRNA was found to be large enough to code for proteins from about 170 kDa to 230 kDa. More recently, using milder deglycosylation methods, a core protein was identified having a molecular weight of about 200 kDa.

Attention has also been devoted to the study and use of the NPGP mucin complex, largely as a result of its high immunogenicity. Thus, a large number of monoclonal antibodies were prepared against it. However, the smaller components of HMFG also appear to be important molecules on the surface of breast epithelial cells. They have a breast specificity as demonstrated by the anti-HME antibodies.

The 46 kDa and 70 kDa HME antigens are found in serum of breast cancer patients and thus can be used as markers for breast cancer in serum assays. In addition, the 70 kDa component has been found to co-purify with the intact mucin complex and has been reported to be associated with the NPGP mucin complex by means of disulfide bonds, making it a possible linker protein of this surface mucin complex.

Few monoclonal antibodies have been prepared against the smaller components of the system. The mucin molecule is apparently more antigenic because of its internally repeated structure. The 46 kDa component of HMFG has been found in the serum of breast cancer patients. Using monoclonal antibodies against the 46 kDa antigen,

circulating immune complexes were found in breast cancer patients and an increase in the circulating 46 kDa antigen was found to be associated with increased tumor burden. The structure of the mucin glycoprotein has recently been determined by cDNA cloning and a partial sequence has been reported for the 70 kDa antigen.

Very little, however, is known about the structure of the 46 kDa antigen and its function, along with the other membrane components, in the normal epithelial cell membrane, milk formation, and breast tumorigenesis. Up to the present time neither the sequences of the about 46 kDalton polypeptide component nor the DNA and RNA encoding it were known.

Disclosure of the Invention

This invention relates to a polypeptide having the antibody binding specificity of the about 46 kDa HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII.

This invention also relates to a fusion protein, comprising

a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; and

a second antigenic polypeptide bound thereto.

Still part of this invention is an antibody having specificity for a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof.

Also provided herein is a method of detecting the presence of a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof in a biological sample, comprising

providing a biological sample suspected of comprising the polypeptide;

adding thereto a polypeptide binding effective amount of an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof under conditions effective to form an antibody-polypeptide complex; and

determining the presence of any complex formed therebetween.

Also part of this invention is a method of determining the presence of epithelial cells in a biological sample, which comprises

providing a biological sample suspected of comprising cells of epithelial origin carrying a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof;

adding thereto a polypeptide binding effective amount of an antibody having specificity for the polypeptide described above, under conditions effective to form an antibody-cell polypeptide complex; and

determining the presence of any complex formed therebetween.

Also provided herein is an in vivo method of imaging cells expressing a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII in a subject, the method comprising

administering to a subject a polypeptide binding effective amount of an antibody having specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof under conditions effective to deliver it to an area of the subject's body suspected of having cells expressing the polypeptide or a functional fragment thereof to form an antibody-cell polypeptide complex;

administering to the subject a detectable label capable of binding to the antibody at a site other than the binding site for the polypeptide; and

detecting the presence of label in the subject's body associated with any complex formed.

Also part of this invention is an in vivo method of vaccinating a subject with a polypeptide having the binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof, the method comprising

administering to a subject a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof in an amount and under conditions effective to vaccinate the subject against the polypeptide, functional fragments thereof or cells carrying the polypeptide or functional fragments thereof.

Yet another method is provided herein for detecting the presence of an antibody having specificity for the about 46 kDalton HMFG differentiation antigen in a biological sample, which comprises

providing a sample suspected of comprising the antibody;

adding thereto an antibody binding effective amount of a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof under conditions effective to form an antibody-polypeptide complex; and

determining the presence of any complex formed therebetween.

This invention also relates to a second method of detecting the presence of an antibody having specificity for the about 46 kDalton HMFG differentiation antigen in a biological sample, comprising

providing a sample suspected of comprising the antibody;

adding thereto an antibody binding effective amount of the fusion protein of this invention under conditions effective to form an antibody-fusion protein complex;

adding thereto a second polypeptide binding effective amount of an anti-second polypeptide antibody under conditions effective to form an antibody-fusion protein-antibody complex; and

determining the presence of any antibody-fusion protein-antibody complex formed therebetween.

Also provided herein is an in vivo method of delivering a therapeutic agent to target cells expressing a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof in a patient, the method, comprising

binding a therapeutic agent to an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof at a site other than the polypeptide binding site; and

administering to a subject suspected of carrying target cells a therapeutically effective amount of the antibody-bound therapeutic agent under conditions effective for reaching the cells' environment; and

allowing for the antibody carrying the therapeutic agent to bind to the cells' polypeptide.

Still part of this invention is an ex vivo method of delivering a therapeutic agent to target cells expressing a polypeptide having the antibody binding specificity of

the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof, comprising

obtaining a biological sample suspected of comprising target cells from a subject;

binding a therapeutic agent to an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or functional fragment thereof at a site other than the polypeptide binding site;

adding the antibody-bound therapeutic agent to the sample under conditions effective to promote the formation of an antibody-cell polypeptide complex; and

returning the sample to the subject.

This invention also relates to a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragments thereof.

Also provided herein is a polyribonucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragments thereof.

Still part of this invention are a polynucleotide and a polyribonucleotide encoding the fusion protein of the invention or antibody binding functional fragments thereof.

This invention also relates to a DNA sequence which is complementary to a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or functional fragments thereof.

This invention also relates to a method of detecting the presence of a polynucleotide sequence encoding a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragments thereof in a sample, the method comprising

providing a sample suspected of comprising the polynucleotide;

melting double stranded polynucleotide present in the sample;

adding thereto a hybridization effective amount of a DNA sequence which is complementary to the polynucleotide encoding a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in labeled form under conditions effective to hybridize any polynucleotide present in the sample having a complementary sequence thereto of at least 15 bases; and

detecting the presence of the DNA-complementary polynucleotide hybrid.

Also provided by this invention is a method of detecting the presence of an RNA sequence encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in a sample, comprising

providing a sample suspected of comprising the RNA;

adding thereto a hybridization effective amount of a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragment thereof in labeled form under conditions effective to hybridize any RNA present in the sample having a complementary sequence of at least 15 bases thereto; and

detecting the presence of the polynucleotide-RNA hybrid.

Also encompassed by this invention is a method of detecting the presence of an RNA sequence encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in a sample, comprising

providing a sample suspected of comprising the RNA;

adding thereto a hybridization effective amount of a polyribonucleotide sequence complementary to that of a polyribonucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragment thereof in labeled form under conditions effective to hybridize any RNA having a complementary sequence thereto of at least about 15 bases; and

detecting the presence of the complementary polyribonucleotide-RNA hybrid.

Also provided herein is a method of detecting the presence of a DNA sequence encoding a polypeptide having the antibody binding specificity of the about 46 kDalton

HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in a sample, comprising

providing a sample suspected of comprising the DNA;

melting double stranded polynucleotides in the sample;

adding thereto a hybridization effective amount of an RNA sequence encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in labeled form under conditions effective to hybridize any DNA present in the sample having a complementary sequence thereto of at least 15 bases; and

detecting the presence of the DNA-RNA hybrid in the sample.

Still part of this invention is a DNA segment comprising an anti-sense sequence to a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof of about 15 to 2000 bases.

Moreover, also provided is a method of treating breast cancer in a subject in need of such treatment, the method comprising administering to the subject a composition comprising a therapeutically effective amount of the anti-sense DNA described above.

This invention also relates to an immunoassay kit comprising, in separate containers

a monoclonal antibody having specificity for a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation

antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof; and

anti-antibody immunoglobulin.

Still part of this invention is an antibody detecting kit comprising, in separate containers

a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof; and

anti-antibody immunoglobulin.

A fusion protein kit is also provided herein which comprises, in separate containers

a fusion protein comprising a polypeptide having the binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragments thereof and a second antigenic polypeptide or fragments thereof which is bound thereto;

an anti-second polypeptide polyclonal or monoclonal antibody; and

anti-antibody immunoglobulin.

Also encompassed by this invention is an anti-breast cancer therapeutic kit comprising, in separate containers

a monoclonal antibody having specificity for a polypeptide provided with the antibody binding specificity of the about 46 kDaltons HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; and

an anti-cancer therapeutic agent selected from the group consisting of immunotoxins and radionuclides.

A more complete appreciation of the invention and many of the intended advantages thereof will be readily

perceived as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying figures.

Brief Description of the Drawing

The figure shows the expression of BA46-1 specific mRNA in human carcinoma cell lines. Total RNA (20ug/lane) was run on a 1.4% agarose gel, blotted, and hybridized to 32P labelled RNA generated from the BA46-1 cDNA clone. The contents of the samples in the different lanes are as follows: a) A549 (lung); b) BT20 (breast); c) ELLG (breast); d) Raji (lymphoid); e) SKBR3 (breast); f) SKOV3 (ovary); g) MDA-MB-361 (breast); h) MDA-MB-331 (breast) i) HeLa (cervix); j) HS578T (breast); k) HT29 (colon); l) PanCl (pancreas); m) MCF7 (breast). Exposure was 16 hours with an intensifying screen.

Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the following discussion.

Best Mode for Carrying out the Invention

This invention arose from a desire to improve on technology useful for detection, diagnosis, and treatment of breast cancer.

This work relies on the isolation of a cDNA clone (BA 46-1) that encodes a portion of an about 46 kDalton polypeptide component of the HMFG system and monoclonal antibodies that bind the about 46 kDalton component of the HMFG system. These monoclonal antibodies have specificity for, and bind to the BA 46-1 cDNA encoded portion of a fusion protein also containing ß-galactosidase and made from the BA 46-1 lambda/gt11 clone.

The nucleotide and deduced amino acid sequence of the BA 46-1 cDNA is shown in Table 1 in Example 6 below. The partial sequence is about 217 amino acids long having a theoretical molecular weight of about 25 kDaltons and, represents the C-terminus of the complete protein. In this sequence, there are 4 potential sites for N-linked glycosylation. The sequence is asparagine and leucine rich. Starting from the C-terminus, the nucleotide sequence extends to the 3' end of the mRNA which contains the AATATA consensus sequence preceeding the poly (A) sequent for cleavage and polyadenylation.

A comparison of the nucleotide sequence to sequences in the EMBL database using FSTNSCAN (PCGENE) revealed extended homology with human serum factors V and VIII, and with protein C. The deduced protein sequence, however, shares identity only with factors V and VIII but not with protein C since the homology at the nucleotide level is found in an intervening sequence (See, Table 2). There is an about 43% identity of the BA 46 to factor V and an about 38% identity to factor VIII. The regions of factors V and VIII shown in Table 2 share an about 47% identity.

The results of the analysis of the deduced amino acid sequence of the about 46 kDalton protein are consistent with that of a glycosylated protein. The function of this protein, however, remains unknown. The homology with the clotting factors may be found in the C1, C2 region of the light chain of factor VIII. Human antibodies that bind this region of the light chain of factor VIII inhibit the factor by preventing its interaction with phospholipids. Since this region of factor VIII has been implicated in phospholipid binding, it is likely that the homologous region in the about 46 kDalton protein may serve a similar role.

The appearance of a shared domain in otherwise different proteins may be due to exon shuffling. The C-terminus may serve as a novel "anchor" sequence for the about 46 kDalton protein or it may be involved in binding of mucin and/or cell membrane to the phospholipids found on the surface of growing milk fat droplets. Alternatively, the homologous sequence may be involved in the assembly of the mucin complex at the plasma membrane surface.

The single stranded RNA probe provided herein is complementary to the ORF found in the cDNA insert. That is, in frame with the ß-galactosidase DNA sequence in the lambda/gt11 vector. This indicates that this ORF represents the sense strand of the BA 46-1 gene since only the complementary strand probe binds to a specific 2.2 kilobase mRNA of ~~ipe~~epithelial cell lines.

The BA 46-1 ß-galactosidase fusion protein expressed by the lambda/gt11 clone is useful for assaying the presence of the about 46 kDalton HMFG polypeptide component or fragments thereof in serum obtained from breast cancer patients. This fusion protein is also useful as an immunogen for generating second generation

monoclonal and polyclonal antibodies. These antibodies may be used, among other applications, to further study the tissue distribution of this antigen and how it relates to the synthesis of its messenger RNAs, to provide improved immunoassays, and to purify and characterize the about 46 kDaltons antigen polypeptide.

Some monoclonal antibodies raised against the about 46 kDalton protein can detect the respective epitopes present on this molecule by radioimmunobinding assays on HMFG membranes and on breast carcinoma membrane material. These monoclonal antibodies do not stain normal breast tissue by immunohistology nor any other normal tissues tested. However, ~~they~~ Some of them weakly stain 24 of 49 breast carcinomas and are negative on all other carcinomas tested. Since some breast carcinomas have very high levels of mRNA for the about 46 kDalton antigenic component, it is possible that antibodies made against the fusion protein have different, and possibly improved, specificity for detecting the about 46 kDalton antigenic component by immunohistophathology.

Northern blots using the cDNA clone in the present work clearly show that the mRNA for this antigen is present in 8 out of 9 breast carcinoma cell lines tested, and in several other non-breast carcinoma cell lines. The RNA for the antigen is, however, present at much lower levels in a lymphoid cell line (Raji). There is considerable variation in the observed expression levels of the about 2.2 kbase RNA detected in the carcinoma cell lines. The lung cells (A549), ovary cells (SKOV3) and two breast cell lines (E11-G and HS578T) accumulate much more of this transcript than other carcinoma cell lines. Overexpression of certain genes, such as Her 2/neu, and the EGF receptor in breast and other carcinomas has been correlated with prognosis. Overexpression of the about

46 kDalton protein in carcinomas may very well correlate with outcome of disease. The about 46 kDalton antigenic component thus shows epithelial specificity. This, however, does not imply that certain epitopes of the molecule may not have greater breast specificity. Moreover, since it is known that there is often a deregulation of expression of many cell antigens associated with malignancy, the expression of this antigen mRNA in non-breast carcinomas does not imply that the antigen is actually expressed nor that the antigen is found in the normal epithelial cells which are counterparts to cells in these epithelial tumors.

Having cloned a portion of the cDNA of this molecule permitted the further deduction of the sequence of the encoded polypeptide. It also permitted the synthesis of recombinant proteins or synthetic peptides from the known amino acid sequence as well as the preparation of a new generation of monoclonal antibodies against specific epitopes of this polypeptide. Also possible with the preparation of the fusion DNA and fusion protein of the invention is the further preparation of polyclonal and monoclonal antibodies against the fusion protein that can be selected to be of greater breast specificity. The HMFG membrane system, in fact, truly represents a purified portion of the apical surface of the normal breast epithelial cell. The about 46 kDalton component being a major molecular species of the HMFG membrane thus also represents a major and perhaps important component of the apical surface of the normal breast epithelial cell.

The cDNA clones of the about 46 kDalton polypeptide component of the HMFG system allowed the deduction of the amino acid sequence of its corresponding polypeptide. These cDNA clones also allowed the preparation of a new

generation of monoclonal antibodies that have sufficient specificity for application to breast cancer immunotherapy, sufficient staining ability in immunohistopathology, for histological evaluation of specificity and prognostic and diagnostic ability, and ability to identify the about 46 kDalton HMFG peptide component or functional fragments thereof in the serum of breast cancer patients, for the construction of serum assays for diagnosis of breast cancer, and the screening for early detection of the disease.

This invention thus provides a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII. In one preferred embodiment the polypeptide has the biological activity of the about 46 kDalton HMFG antigen molecule, and more preferably the polypeptide comprises the 46 kDalton HMFG differentiation antigen or an antibody binding functional fragment thereof.

The polypeptide of the invention may be about 90 to 500 amino acids long, preferably about ~~110~~ 200 to ~~280~~ 450 amino acids long, and more preferably about ~~200~~ 220 to ~~250~~ 420 amino acids long.

In another preferred embodiment the polypeptide has the amino acid sequence shown in Table 2 or an antibody binding functional fragment thereof, preferably of about 5 to 100 amino acids long, and more preferably 15 to 50 amino acids long. Particularly preferred are amino acid sequences which correspond to the specific epitopes which are recognized by anti 46 kDalton HMFG differentiation antigen antibodies.

Also provided herein is a pharmaceutical composition, which comprises

10/31/90

an antibody binding effective amount of the polypeptide described above; and

a pharmaceutically acceptable carrier.

This pharmaceutical composition is intended for animal, including human, administration. Each dose preferably contains about 0.1 to 1000 mg of the polypeptide, and more preferably about 10 to 500 mg. Any pharmaceutically acceptable carrier can be utilized for the preparation of the composition. Examples of suitable carriers and other additives are flavorings, preservatives, colorants, salt solutions such as saline, oils or solids, among others. However, any liquid or solid carrier which does not hydrolyze the polypeptide is suitable. The pharmaceutical composition as well as the polypeptide itself are best kept under refrigeration and/or frozen. The polypeptide and the pharmaceutical composition may be vacuumed dried and packaged in a sterile container for transportation to their destination. The composition may comprise about 0.01-99.99 wt% of the polypeptide, and preferably about 0.1-10 wt%, the remainder being the carrier.

Also provided herein is a fusion protein, which comprises

the polypeptide described above; and

a second antigenic polypeptide or an antibody binding functional fragment thereof which is operatively linked or bound to the polypeptide of the invention.

The fusion protein may generally be composed of an antibody binding functional fragment of the polypeptide of the invention bound to an antibody binding functional fragment of the second antigenic polypeptide, which are about ~~50~~ to 700 amino acids long and 10 to ~~1100~~ 500 amino acids long, respectively, and preferably about 15 to 100 amino acids long and 200 to 400 amino acids long,

respectively. However, other sizes of the polypeptides, and/or fragments thereof, either larger or smaller, may be utilized as long as their antibody binding capability is preserved.

Any polypeptide is suitable as the second antigenic polypeptide as long as it acts as an antigen to elicit the formation of antibodies by a mammal as is known in the art. The second antigenic polypeptide may be chosen in addition because it possesses some other property which is of use for the identification and/or use of the fusion protein. By means of example the second antigenic polypeptide may be a protein such as ß-galactosidase or a functional fragment thereof. However, any other second antigenic polypeptide may be utilized as long as antibodies to it can be raised. For example, gene 10 from bacteriophage T7 may also be used.

Both, the polypeptide of the invention and the fusion protein may be prepared by methods known in the art. By means of example, the polypeptide may be prepared synthetically or it may be produced by the expression of a DNA fragment that encodes it which can be cloned into a vector and inserted into a host capable of expression. (Marston, F.A., in DNA cloning: A practical approach, Glover, P., ed., IRL Press, London, Vol. 3, pp. 59-88 (1987)). The fusion protein may be prepared by providing a recombinant DNA containing sequences which encode the amino acid sequences of the two polypeptides. This DNA may be cloned into a vector and expressed in a host.

Also part of this invention is an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII.

Methods for raising antibodies are known in the art and need not be described herein. Particularly preferred are antibodies which are monoclonal antibodies. Methods of preparing monoclonal antibodies against a specific polypeptide are also known in the art and need not be described in detail herein.

The antibodies raised against the biologically pure polypeptide or fragments thereof have increased affinity and/or specificity for the polypeptide. Typically, the affinity may be about 10-8 to 10-5, and in some cases greater than 10-8.

In a particularly preferred embodiment of the invention the antibody also has affinity for the C1 and/or C2 regions of clotting factor VIII (light chain). Still another preferred embodiment is that wherein the antibody of the invention is the Fab fragment thereof, with its binding capacity preserved. Also, preferred are a single chain of the antibody, or the Fab fragment having the described functionality and functional fragments thereof.

Also provided herein is a pharmaceutical composition, which comprises

a polypeptide binding effective amount of an antibody having an affinity of about 10-10 to 10-5, for a polypeptide provided with the antibody binding specificity of the about 46 kDalton differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII of the HMFG system; and

a pharmaceutically acceptable carrier.

Typically, the antibody is provided in an amount of about 0.001 to 10,000 mg, and more preferably about 10 to 500 mg. Any pharmaceutically acceptable carrier is suitable as indicated above. Other ingredients may also

be contained in the composition such as radionuclides, chemotherapeutic drugs, interferon, toxic agents such as ricin A-chain, abrin A-chain, saline salt solutions, preservatives, flavors, colorants and buffers, among others, as is known in the art. The preparation of the pharmaceutical composition can be undertaken as is known in the art by admixing the polypeptide or the antibody with the pharmaceutically-acceptable carrier in the absence of hydrolyzing conditions, then vacuum dried and packaged in a sterile container or provided as a sterile solution.

Also part of this invention is a method of detecting the presence in a biological sample of a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof, comprising

providing a biological sample suspected of comprising the polypeptide;

adding thereto a polypeptide binding effective amount of an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; under conditions effective to form an antibody-polypeptide complex; and

determining the presence of any complex formed.

This method is suitable for detecting the presence of the polypeptide in biological samples such as animal cells, cell extracts or body fluids. Typically, any body fluids are encompassed herein. Examples are serum, plasma, urine, breast fluid, tissue biopsies, and fine needle aspirates.

The sample may be previously treated, e.g., to avoid interference by metals, non-specific proteins, fats, nucleic acids, and the like.

The biological sample may also be diluted in order that the content of the polypeptide be in a range of about 0.0001 to 10 mg/ml, and more preferably about 0.001 to 0.1 mg/ml. The antibody may be added as known in the art in an amount of about 0.0001 to 1.0 mg/ml of sample, and more preferably about 0.001 to 0.1 mg/ml of sample.

Other conditions for the assay may be as follows. The sample may be homogenized and centrifuged to remove particulate material and fatty material. Detergents may be added to dissolve membranes, solubilize fatty material and reduce background. Also added may be carrier proteins such as bovine serum albumin to reduce non-specific binding of the antibodies, and chelators to remove interfering divalent metal ions.

The determination of the presence of any complex formed between the antibody and the polypeptide may be done by a variety of methods known in the art. By means of example will be cited herein the further addition of a labeled anti-antibody immunoglobulin to form a double antibody-polypeptide complex which is labeled. The label may be a radiolabel, a fluorescent label, an enzyme label or biotin to be later detected as a conjugate of avidin, streptavidin or magnetic bead. After this step the amount of label bound to the complex may be assessed by methods known in the art.

Also provided herein is a method of determining the presence in a biological sample of epithelial cells, which comprises

providing a biological sample suspected of comprising cells of epithelial origin carrying a polypeptide having the antibody binding activity of the

about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof;

adding thereto a polypeptide binding effective amount of an antibody having specificity for a polypeptide having the antibody binding specificity of the about 46 kDalton differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII of the HMFG system under conditions effective to form an antibody-cell polypeptide complex; and

determining the presence of any complex formed.

This method is particularly well suited for biological samples such as bone marrow samples. However, it may be practiced with samples of other origin as well. The steps are in general conducted as described above and the determination of the presence of epithelial cells may be done by the identification, either qualitative or quantitative, of any complex formed with the antibody as already described.

The detection may also be undertaken by assaying for the presence of ribonucleic acid (RNA) encoding the about 46 kDalton protein using nucleic acid probes based on sequences such as the one shown in Table 1 and methods known in the art such as PCR (Erlich, H.A., in PCR Technology: Principles and Applications for DNA Amdification, 1989, Stockton Press).

Also provided herein is an <u>in vivo</u> method of imaging cells expressing a polypeptide having the antibody binding specificity of the about 46 kDalton differentiation antigen of the HMFG system and/or homology to at least one of the light chains of clotting factors V and VIII in a subject, the method which comprises

administering to a subject a polypeptide binding effective amount of an antibody having specificity for a polypeptide with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII under conditions effective to deliver it to an area of the subject's body suspected of having cells expressing the polypeptide to form an antibody-cell polypeptide complex;

administering to the subject a detectable label capable of binding to the antibody at a site other than binding site of the polypeptide; and

detecting the presence of the label associated with any complex formed in the subject's body.

The administration of the antibody may be at a concentration of about 0.5 to 50 mg/ml, and more preferably about 5 to 20 mg/ml. A total of about 10 to 50 ml of the antibody composition may be given at any one particular time. The regimen of administration may be a single dose or the antibody may be administered in a continuous manner in order to continuously suppress the presence of polypeptide or functional fragments thereof in the subject's cells. Thus, repeated doses of the antibody composition are also contemplated.

The antibody may be administered in a pharmaceutical composition as described above, or in any other form found suitable. The administration of the antibody may be conducted by the intravenous, intraperitoneal, intracavitary, lymphatic, intratumor and intramusculary routes, among others. Other routes as suitable may also be utilized which will not hydrolyze the peptide links of the antibody.

The administration of a detectable label may be conducted by providing an anti-antibody immunoglobulin or

a binding-functional fragment thereof which is labeled and then detecting the amount of label bound to the complex. These technologies are known in the art and need not be further described herein.

Also provided herein is a method of detecting a presence in a biological sample of an antibody having affinity for the about 46 kDalton HMFG differentiation antigen, comprising

providing a sample suspected of comprising the antibody;

adding thereto an antibody binding effective amount of a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII under conditions effective to form an antibody-polypeptide complex; and

determining the presence of any complex formed.

The method described above utilizes the polypeptide of the invention in order to detect the presence of antibodies in a mammal generated as a consequence of the presence of such polypeptide in the mammal's body. The sample may be treated as indicated above to eliminate interference of other proteins and/or components of the sample. In the case of blood, serum may be obtained first, and then the serum may be treated as follows.

Normal human or bovine serum may be added, and/or bovine serum albumin (BSA) is used as a blocking agent to reduce non-specific reactivity.

The polypeptide is added to the sample in an amount of about 0.00001 to 1.0 mg/ml of sample, and more preferably about 0.0001 to 0.1 mg/ml of sample. However, other amounts may also be utilized as seen suitable. The amount of antibody in the sample may be controlled by dilution. Optimal ranges of antibody in the sample are

about 0.00001 to 0.1 mg/ml; and more preferably about 0.0001 to 0.01 mg/ml. However, other amounts may also be utilized. The steps of this method are practiced as described above, including the determination of the presence of antibody-polypeptide complex. The conditions for the assay are in general those known in the art, such as pH temperature and the like.

Also provided herein is a method of detecting the presence of an antibody having affinity for the about 46 kDalton HMFG differentiation antigen in a sample, the method comprising

providing a sample suspected of comprising the antibody;

adding thereto an antibody binding effective amount of a fusion protein comprising a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII and a second antigenic polypeptide or an antibody binding functional fragment thereof bound to one another under conditions effective to form an antibody-fusion protein complex;

adding thereto a second polypeptide binding effective amount of an anti-second polypeptide antibody under conditions effective to form an antibody-fusion protein-antibody complex; and

determining the presence of any antibody-fusion protein-antibody complex form.

As in the case of the previous methods this method is practiced preferably with a monoclonal antibody. The amounts of antibody added to the sample are preferably about 0.00001 to 0.1 mg/ml sample, and more preferably about 0.0001 to 0.01 mg/ml of sample. However, other amounts may also be utilized. As in the previous cases

the sample may be pretreated prior to the addition of the fusion protein. One example is the dilution of the sample and the elimination of interfering components. These steps are undertaken as is known in the art and need not be further described herein.

Also provided herein is an in vivo method of vaccinating a subject with a polypeptide having the binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a functional fragment thereof, comprising

administering to a subject to be vaccinated a polypeptide having the antibody binding specificity and/or homology to at least one of the light chains of clotting factors V and VIII of the above 46 kdalton HMFG differentiation antigen or a functional fragment thereof in an amount and under conditions effective to vaccinate the subject against the polypeptide, functional fragments thereof or cells carrying the polypeptide or functional fragments thereof. This in vivo method may be utilized to vaccinate a cancer patient against a polypeptide of the described characteristics or cells carrying it. In this manner the patient is induced to raise an immune response against the polypeptide or cells carrying the polypeptide.

The vaccinating polypeptide may be administered to the subject in an amount of about 0.1 to 100 mg/ml, and more preferably about 2 to 50 mg/ml. Typically, any dose will consist of about 0.1 to 50 ml of the vaccinating polypeptide, and more preferably about 2 to 10 ml. The vaccinating agent may be administrated in a single dose or it may be administered on a continuous basis for periods of up to about 6 months, and sometimes in excess of one year. More prolonged periods of time are also encompassed for vaccination according to this invention.

Also provided herein is an in vivo method of delivering a therapeutic agent to target cells expressing a polypeptide having the antibody binding activity and/or homology to at least one of the light chains of clotting factors V and VIII of the about 46 kDalton HMFG differentiation antigen in a patient, comprising

binding to a monoclonal antibody having specificity for a polypeptide provided with the antibody-binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII a therapeutic agent at a site other than the polypeptide binding site;

administering to a subject suspected of carrying the target cells a therapeutically effective amount of the antibody-bound therapeutic agent under conditions effective to deliver the agent to the cells' environment; and

allowing for the antibody carrying the therapeutic agent to bind to the cells' polypeptide to permit therapeutic agent to exert its effect on the cells.

This in vivo method may be utilized for treating cancer patients that are afflicted with cancer to epithelial cells, e.g., breast cancer.

The therapeutic agent may be any anti-cancer agent known in the art. Examples of therapeutic agents are radionuclides, chemotherapy drugs, toxic agents such as ricin A-chain, abrin A-chain, etc. However, others may also be utilized. The therapeutic agent is bound to the antibody by means known in the art. More specifically, a radionuclide such as 131I is bound to the antibody by oxidation of amino acids such as tyrosine, or 90Y attached via a chelator, and the conjugate injected intravenously or intraperitoneally into humans carrying human breast tumors, and the growth of the tumor is thus

inhibited. (e.g., for mice, Ceriani, et al, Cancer Res. 48:4664-4672(1988)).

The antibody-bound therapeutic agent may be administered to the subject in an amount of about 1 to 100 mg of composition/ml, and more preferably about 2 to 20 mg of composition/ml. Typically, any dose will consist of about 1 to 50 ml of antibody-bound therapeutic agent containing composition and more preferably about 2 to 10 ml. The therapeutic agent may be administered as an antibody-bound agent in a single dose or it may be administered on a continuous bases for periods of up to about 6 months, and sometimes in excess of one year. More prolonged periods of time are also encompassed for treatment herein.

Also provided herein is an ex vivo method of delivering a therapeutic agent to target cells expressing a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII, the method which comprises

obtaining from a subject a biological sample suspected of comprising target cells;

binding to a monoclonal antibody having specificity for a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII a therapeutic agent at a site other than the polypeptide binding site;

adding the antibody-bound therapeutic agent to the sample under conditions effective to promote the formation of an antibody-cell polypeptide complex;

allowing the agent to exert its effect on the cells;

and returning the sample to the subject.

The non-conjugated antibody may also be added to the sample in the presence of complement, which causes lysis of the cells, prior to returning the sample to the subject.

In general the steps of this method may be practiced as described above, particularly in terms of the preparation of the biological sample, and binding of the therapeutic agent to the antibody as well as the addition of the antibody-bound therapeutic agent to the sample. With respect to the return of the sample to the subject, this may be done by means known in the art. For example, the already treated sample may be returned to a subject's body in sterile form by the intravenously, intracavitary, intraperitoneal, and intratumor routes. However, other routes known in the art may also be utilized.

Also provided herein is a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen of the invention and/or homology to at least one of the light chains of clotting factors V and VIII or binding functional fragments thereof. The polynucleotide is provided either as a double stranded DNA or as a single stranded DNA containing the coding strand of the polynucleotide. The fragments of the polynucleotide may be of about 15 to 2000 bases, and more preferably about 30 to 300 bases.

Also provided herein is a DNA sequence which is complementary to the coding strand of the polynucleotide described above.

Both the double stranded and the single stranded DNAs discussed above are also provided in labeled form. The labeling may be conducted as is known in the art with radioactive atoms such as 32P,14C, 3H, and the like. However, other radionuclides may also be utilized.

Particularly preferred is a polynucleotide having the DNA sequence shown in Table 1 of this patent or fragments thereof or DNA sequences comprising about 9 to 2000 bases, and more preferably about 18 to 200 bases. However, fragments of other sizes may also be utilized and are encompassed herein.

Also part of this invention is a polyribonucleotide encoding a polypeptide having the antibody binding specificity and/or homology to at least one of the light chains of clotting factors V and VIII of the about 46 kDalton HMFG differentiation antigen or fragments thereof. This is the coding RNA for the polypeptide.

The polyribonucleotide sequences may be of a size of about 9 to 3000 bases long, and more preferably fragments of about 18 to 300 bases long. However, other fragment sizes are also encompassed herein.

Still part of this invention is a non-coding strand of a polyribonucleotide having a sequence complementary to that of the polyribonucleotide described above. This polyribonucleotide sequence is capable of hybridization to the coding RNA strand or to the non-coding strand of the corresponding DNA. In a particularly preferred embodiment the polyribonucleotide is provided in labeled form.

Also part of this invention is a polynucleotide encoding a fusion protein comprising a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII and a second antigenic polypeptide or an antibody binding functional fragment thereof bound to one another.

The polynucleotide may be about 400 to 4000 bases long, and more preferably about 500 to 1,400 bases long. However, other size polynucleotides are also encompassed herein.

Also provided herein is a polyribonucleotide encoding a fusion protein comprising a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII and a second antigenic polypeptide, or an antibody binding functional fragment thereof bound to one another. Also, a polyribonucleotide is provided which is complementary to the sequence of the RNA encoding the fusion protein.

The polyribonucleotide encoding the fusion protein may be about 400 to 4000 bases long, and more preferably about 500 to 1,400 bases long. Fragments thereof may be about 9 to 100 long, and more preferably about 15 to 70 bases long.

Still part of this invention is a polynucleotide encoding the fusion protein of the invention or functional fragments thereof about 15 to 4000 bases long, and more preferably about 50 to 1800 bases long. The polynucleotide encoding the fusion protein is provided as a double stranded DNA or as a single stranded DNA which encompasses the coding strand of the fusion protein and a second polynucleotide encompassing a sequence corresponding to the non-coding DNA strand or fragments thereof. The latter polynucleotide provided herein is a polynucleotide comprising DNA sequences complementary to the polynucleotide encoding the fusion protein. Both the DNA and RNA sequences encoding the fusion protein may be provided in labeled form. Particularly useful labels are 32P and others known in the art. The DNAs and RNAs are labeled by methods known in the art.

Also provided herein is the method of detecting the presence in a sample of the polynucleotide sequence encoding a polypeptide having the antibody binding

activity of the about 46 kDaltons HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII, the method comprises

providing a sample suspected of comprising the polynucleotide;

melting double stranded polynucleotide present in the sample;

adding thereto a hybridization effective amount of a DNA sequence which is complementary to the coding strand of a polynucleotide encoding the polypeptide of the invention in labeled form under conditions effective to hybridize any polynucleotide having a complementary sequence of at least 15 bases thereto; and

detecting the presence of the DNA-complementary polynucleotide hybrid.

The sample subjected to this method may be a biological sample or it may be a sample generated in the laboratory. If the sample contains cells where the polynucleotide is located, the cells need to be lysed, and optionally the DNA isolated from the remainder materials. This is done by methods known in the art.

The sample may be further diluted and/or prepared for the melting of double stranded polynucleotide sequences present therein. The melting step is conducted as is known in the art. In general, the sample is prepared by lysing the cells in 4M guanidinium isothiocyanate to denature protein and prevent RNAse activity. Extracts are run on a Cesium Chloride density step gradient ultracentrifugation where RNA, DNA and protein are separated according to their relative densities. DNA and RNA are further purified by extraction with organic solvents, and concentrated by precipitation in 70% ethanol. (Sambrook et al, in

Molecular Cloning: A Laboratory Manual, Second edition, Cold Spring Harbor Press, N.Y., (1989)). Melting is accomplished by raising the temperature of the sample about 20°C over the Tm of the DNA, or by raising the pH to above 12.

To the melted DNA is added a hybridization effective amount of labeled DNA complementary to the coding strand of a polynucleotide encoding a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII. The conditions for suitable hybridization of DNA-DNA segments are known in the art. The degree of stringency is determined by the number of complementary sequences desired to be hybridized. In general when more stringent conditions are utilized hybridization will occur with DNA sequences which have a higher degree of complementarily with the probe. Thus, when a low degree of stringency is desired to detect sequences with low complementarily, the conditions may be varied accordingly. In general, the conditions may be as follows.

The general conditions may be varied but are generally as follows. The sodium ion concentration is about 1M, the pH about 5-9, the temperature about 65°C or about 20°C below the melting temperature of the duplex DNA of the probe sequence and its complementary strand (Britten, R. et al, Methods in Enzymology 29:363(1974); Sambrook et al, supra).

The DNA-complementary polynucleotide labeled hybrid may be detected by methods known in the art. Typically, the double stranded DNA is restricted with enzymes and run on an electrophoresis gel to separate the different size strands. The gel is blotted onto a specially prepared filter, hybridized, and the filter is then

exposed to a photographic plate for a period of time effective to obtain a picture thereof. The plate is then developed and the different fragments analyzed.

For a more qualitative detection of the presence of the double stranded labeled hybrid, the unrestricted DNA may be blotted onto a filter, hybridized, exposed to a photographic plate and the plate developed to merely detect the presence of radiolabel.

Also provided herein is a method of detecting the presence of an RNA sequence encoding a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof in a sample, comprising

providing a sample suspected of comprising the RNA;

adding thereto a hybridization effective amount of the coding strand of a labeled polynucleotide encoding a polypeptide with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII in single stranded form under conditions effective to hybridize any RNA having a complementary sequence of about at least 15 bases thereto; and

detecting the presence of the polynucleotide-RNA hybrid.

In essence, the above method is conducted in a manner similar to the previously described method of detecting the presence of a DNA sequence, with the additional precaution of substantially ensuring a lack of degradation of the RNA contained in the sample. In general, the following must be additionally done when detecting RNA.

The use of RNAse inhibitors and the pretreatment of labware with diethylpyrocarbonate to inactivate any contaminating RNAses. Hybridizations are conducted generally at a higher stringency because RNA:RNA hybrids are more stable than DNA:DNA hybrids. For example, the hybridization may be conducted at 65°C in 50% formamide. The Tm of DNA duplexes is reduced by about 0.72°C per 1% formamide added. (See, Sambrook et al, supra; Casey J. and Davidson N., Nucl. Acids Res. 4:1539-1552(1977)).

If the RNA is contained inside the cells, the cells must be lysed to expose the ribonucleic acid. This is done by means known in the art such as detergent lysis, which may be followed by treatment with proteases.

Also part of this invention is a method of detecting the presence in a sample of an RNA sequence encoding a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or a fragment thereof, the method comprising

providing a sample suspected of comprising the RNA;

adding thereto a hybridization effective amount of a labeled oligoribonucleotide complementary to at least a portion of a polyribonucleotide sequence encoding a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII under conditions effective to hybridize thereto RNA having a complementary sequence of at least about 15 bases; and

detecting the presence of the polyribonucleotide-RNA hybrid.

This method is in general practiced in a manner similar to the two previous methods except that in this

case precautions must be taken not to permit any degradation of the RNA sequences present in the sample and the probe. The conditions for RNA-RNA hybridization are known in the art. In general, the conditions utilized involve a temperature of about 65°C and about 50% formamide (mentioned above).

When the RNA is contained inside cells, the cells must be lysed to permit the exposure of the RNA.

A method of detecting the presence in a sample a polynucleotide sequence encoding a polypeptide having the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII or fragments thereof is also part of this invention. The method comprises

providing a sample suspected of comprising the polynucleotide;

melting double stranded polynucleotide present in the sample;

adding thereto a hybridization effective amount of a labeled RNA sequence encoding a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII under conditions effective to hybridize thereto any polynucleotide having a complementary sequence thereto of at least about 15 bases; and

detecting the presence of the RNA-complementary polynucleotide hybrid.

When the polynucleotide is inside the cells, the cells may be lysed to expose the DNA.

Also part of this invention is a DNA segment comprising an anti-sense sequence to the coding strand of a polynucleotide encoding a polypeptide having the

antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII of about 200 to 3,000 nucleotides. More preferably, the DNA segment may have about 100 to 1,000 nucleotides.

The concept of anti-sense sequences is known in the art. Synthetic oligonucleotides may be prepared that are complementary to the messenger RNA encoding a target protein. The oligonucleotide or a chemically modified equivalent thereof are added to cells. The oligonucleotide binds the target mRNA and thus inhibits the translation of the target protein. (Markus-Sekura C.J., Techniques for using Antisense Oligonucleotides to Study Gene Expression, Analytical Biochemistry 172:289-295(1988)).

Alternatively, antisense-RNA is used to block translation of sense RNA. The antisense RNA is generated from a viral or plasmid DNA vector that contains a copy of the target gene situated in the reverse orientation with respect to the direction of transcription. A virus may be used as a carrier to introduce the inverted gene into the target cell genome. (Izant, J.G. and Weintmub H., Science 229:345-352(1985)).

Fragments of the anti-sense DNA segment are also provided herein and they may comprise about 15 to 100 bases, and more preferably 30 to 50 bases. The anti-sense sequences may be obtained by methods known in the art such as the following.

Antisense oligonucleotides can be made by modifying their phosphate moiety to increase biological lifetime, to enhance permeability into cells and to strengthen binding to target. For example, oligomethylphosphonates (Miller, P.S., Reddy, M.P., Murakami, A., Blake, K.R., Lin, S.B. and Agris, C.H. (1986) Biochemistry

25:5092-5097), or oligophosphorothionates (LaPlanche, L.A., James, T.L., Powell, C., Wilson, W.D., Uznanski, B., Stec., W.J., Summers, M.F. and Zon, G. (1986) Nucleic Acids Res. 14:9081-9093). Alternatively, the target gene may be inserted into a viral-based eukaryotic expression vector in reverse orientation and introduced into mammalian cells (See, Sambrook, J. et al, supra).

Also part of this invention is a pharmaceutical composition which comprises a therapeutically effective amount of an anti-sense DNA sequence to the coding strand of a polynucleotide encoding a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen or a fragment thereof; and

a pharmaceutically acceptable carrier.

The composition may be provided in different amounts. Typically, the anti-sense DNA will be provided in an amount of about 0.01 to 99.99 wt% and more preferably about 0.1 to 20 wt%, the remainder being carrier and/or other known additives. The pharmaceutically acceptable carrier may be any carrier which does not degrade DNA. Examples of carriers and other additives are buffered saline solution, human serum albumin and the like. However, others may also be utilized. The pharmaceutical composition may be prepared by admixing the anti-sense DNA with the carrier as is known in the art, freeze dried and packaged in a sterile container. The composition may be maintained refrigerated and/or frozen.

A method of treating breast cancer in a subject in need of such treatment is provided with this invention. The method comprises administering to a subject a composition comprising a therapeutically effective amount of an anti-sense DNA sequence to the coding strand of a polynucleotide encoding a polypeptide having the antibody

binding specificity of the about 46 kDalton HMFG differentiation antigen or a fragment thereof.

This method may be practiced by administering an amount of about 5 to 800 mg anti-sense DNA, and more preferably about 20 to 200 mg anti-sense DNA in a pharmaceutical composition. The composition may be administered by a parenteral, intravenous or intrabreast route. However, other routes of administration may also be utilized.

Part of this invention is also an immunoassay kit comprising, in separate containers

a monoclonal antibody having specificity for a polypeptide provided with the antibody binding activity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; and

anti-antibody immunoglobulin.

This immunoassay kit may be utilized for the practice of the various methods provided herein. The monoclonal antibody and the anti-antibody immunoglobulin may be provided in an amount of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The anti-antibody immunoglobulin may be a polyclonal immunoglobulin, protein A or protein G or functional fragments thereof, which may be labeled prior to use by methods known in the art.

Also provided herein is an antibody detecting kit comprising, in separate containers

a polypeptide having the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; and

anti-antibody immunoglobulin.

The anti-antibody immunoglobulin may be labeled prior to use.

Also provided herein is a fusion protein kit comprising, in separate containers

a fusion protein comprising a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII and a second antigenic polypeptide or an antibody binding functional fragment thereof bound to one another;

an anti-second polypeptide monoclonal antibody; and

anti-antibody immunoglobulin.

The fusion protein may be provided in an amount of about 0.001 mg to 100 grams in sterile form, and more preferably about 0.01 mg to 1 gram. The anti-second polypeptide monoclonal antibody may also be provided in sterile form in an amount of about 0.001 mg to 100 grams, and more preferably about 0.01 mg to 1 gram. The anti-antibody immunoglobulin may be provided in a separate container in an amount of about 0.001 mg to 100 grams and more preferably about 0.01 mg to 1 gram. The entire kit may be packaged for shipping and storage.

Also provided herein is an anti-breast cancer therapeutic kit comprising, in separate containers

a monoclonal antibody having specificity for a polypeptide provided with the antibody binding specificity of the about 46 kDalton HMFG differentiation antigen and/or homology to at least one of the light chains of clotting factors V and VIII; and

an anti-cancer therapeutic agent selected from the group consisting of immunotoxins and radionuclides.

The monoclonal antibody is provided in an amount of about 1 to 20 grams, and more preferably about 2 to 10

grams in sterile form. The antibody may be freeze- dried and packaged. The therapeutic agent may be any known anti-cancer therapeutic agent. By means of example the agent may be abrin-A chain, ricin A-chain, immunotoxins, chemotherapy drugs and 131I and 90Y radionuclides, among others.

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any embodiment thereof, unless so specified.

EXAMPLES

Example 1: Immunoscreening lambda/gt11 cDNA library

A human breast cDNA library was purchased from Clontech (Palo Alto, CA). The library was prepared from RNA extracted from adult breast tissue excised during mastectomy, during 8th month pregnancy, showing well-differentiated tissue and lactational competence. The oligo-dT primed cDNA from this tissue was inserted into the Eco R1 site of lambda/gt11. Plating and screening of the library with MoAbs were done essentially as described by Young and Davis (Young, R.A. and Davis, R.W. (1983), Proc.Nat'l Acad. Sci. U.S.A., 80, 1194-1198). The library was screened with a cocktail of MoAbs Mc3, Mc8, Mc15 and Mc16 ~~[Reference missing 1]~~, all of which bind the 46 kDa component of human milk fat globule.

Peterson, J.A. et al. Hybridoma 9: 221-235, 1990.

Example 2: Blot Analysis

Cell lines were grown to late log phase and total cell RNA prepared by the method of Chirgwin et al (Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Biochemistry, 18, 5294-5299. RNA was glyoxalated, electrophoresed, and blotted according to Thomas (Thomas, P. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Nat'l. Acad. Sci. U.S.A., 77, 5201-5205) and RNA bound to nylon (Biodyne) filters using UV irradiation.

Single stranded RNA probes were made in vitro, using SP6 and T7 RNA polymerase according to manufacturer (Promega) and labelled by incorporation of [32P] UTP at 800 Ci/mmol (Amersham). Hybridization of RNA probes to RNA blots was at 70°C, 0.1 x SSC, 0.1% SDS. Blots were exposed to X-ray film (Kodak X-AR) at -80°C with intensifying screens.

10/31/90

Example 3: DNA Sequencing

Large scale bacteriophage DNA preparations were made from phage lysates, and the Eco R1 digested cDNA insert subcloned into pGEM3 (Promega, Madison, WI) according to standard protocols (Sambrook, J., Fritsch, D., and Maniatis, T. (1990) Molecular Cloning: A Laboratory Manual/Second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Dideoxy sequencing of the insert in pGEM3 was done with a modified T7 DNA polymerase (Sequenase) directly on the plasmid DNA using T7 or SP6 promoter sequence primers (Promega) according to the manufacturer's protocol (USB, Cleveland, OH). The sequence was confirmed by sequencing both strands of the insert.

Example 4: Results

15 positive plaques were selected after screening about 1 x 10^6 plaques from lambda/gt11 lactating breast cDNA library. The largest cDNA, BA46-1 was 1271 base pairs long. A second cDNA clone gave 3' end sequence data extending to 1384 base pairs, including the polyadenylation site. A series of positive lambda/gt11 clones were used to lysogenize Y1089 and the resulting fusion protein contained in induced cell extracts were analyzed by dot blot analysis for reactivity with each of the monoclonal Abs contained in the screening cocktail.

It was found that Mc8, Mc15 and Mc16 bound to all the positive lambda/gt11 lysogen extracts but not to control lambda/gt11 extract (not shown). Mc3, however, did not bind any of the lysates indicating that its epitope requires glycosylation, secondary structure, or is not present in the library.

Example 5: Study of RNA Sequence

Single stranded RNA probes representing each strand of the BA46-1 cDNA insert were prepared by subcloning into Gem3 and transcribing in vitro with T7 or SP6 polymerase.

Several carcinoma cell lines were studied including 5 breast lines and a lymphoid cell line for BA46-1 specific RNA. As shown in the figure accompanying this patent, a single 2.2 kb RNA was readily detected in most carcinoma cell lines tested. This RNA is also detectable in Raji, SKBR3 and PANC1, but at much lower levels requiring longer exposures than shown in the figure.

There was considerable variation in the observed expression levels of the 2.2 kb RNA that were detected in the carcinoma cell lines. The lung (A549), ovary (SKOV3) and two breast (E11-G and HS578T) cell lines accumulated from 10-50 fold more of this transcript than the other carcinoma cell lines. It should be noted that overexpression of certain genes, such as Her 2/neu and EGF receptor in breast and other carcinomas has previously been correlated with prognosis. (Slamon, D.J., Goldophin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., and Press, M.F. (1989) Science 244, 707-712.)(Dickson, R.B., Bates, S.E., McManaway, M.E., and Lippman, M.E. (1986) Cancer Res., 46, 1707-1713).

Example 6: Specificity Studies

Although the antibodies used to select the cDNA had specificity for breast carcinomas (Peterson, J.A., et al., Hybridoma 9:221-235 (1990)), expression of the about 2.2 kb RNA fragment that encodes the about 46 kDa protein occurs in many different carcinoma cell lines. A lack of breast specificity found may be attributed to a

de-regulation of this gene in carcinomas but not in normal tissue. Alternatively, normal epithelial tissue may express the about 46 kDa protein but process it in a way that blocks the epitopes that are exposed in the breast cell version of the protein by, for example, alterations in glycosylation.

The high molecular weight mucin-like protein of HMFG is also expressed in non-breast carcinomas but its altered processing in the pancreas, for example, leads to exposure of different antigenic sites than in the breast (Lan, M.S., Hollingworth, M.A., and Metzgar, T.S. (1990) Cancer Res., 50, 2997-3001).

Example 7: Study of DNA Sequence

The nucleotide and derived amino acid composite sequence of Ba46-1 and Ba46-2 cDNAs is shown in Table 1 below.

12/31/90

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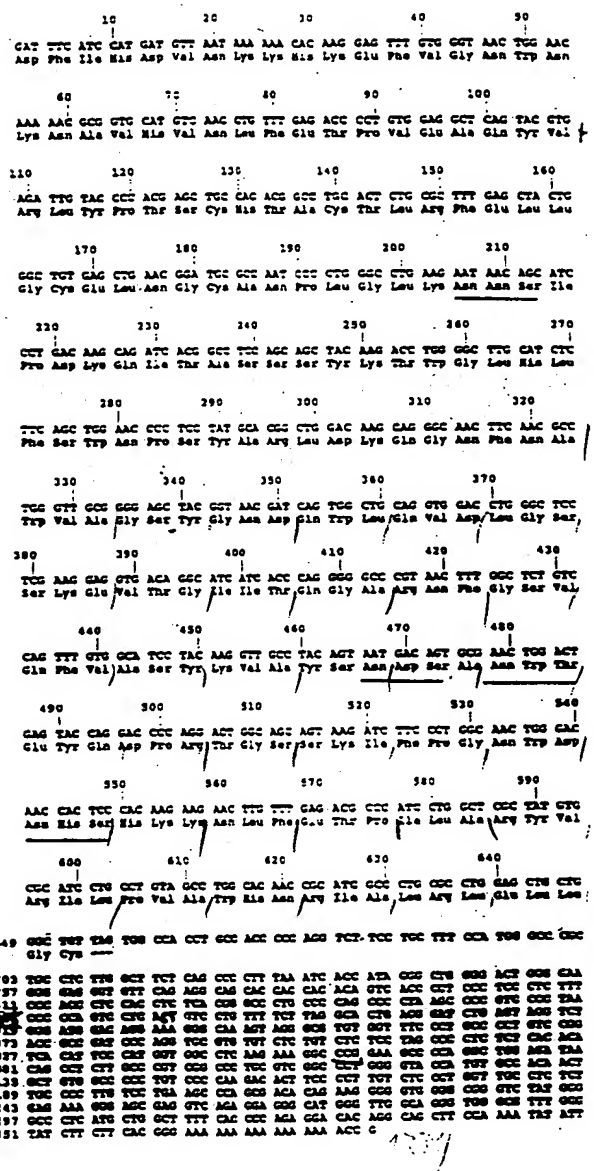
Table 1: DNA Sequence and Derived Amino Acid Sequence of BA46-1 cDNA



Potential n-linked glycosylation sites are underlined.

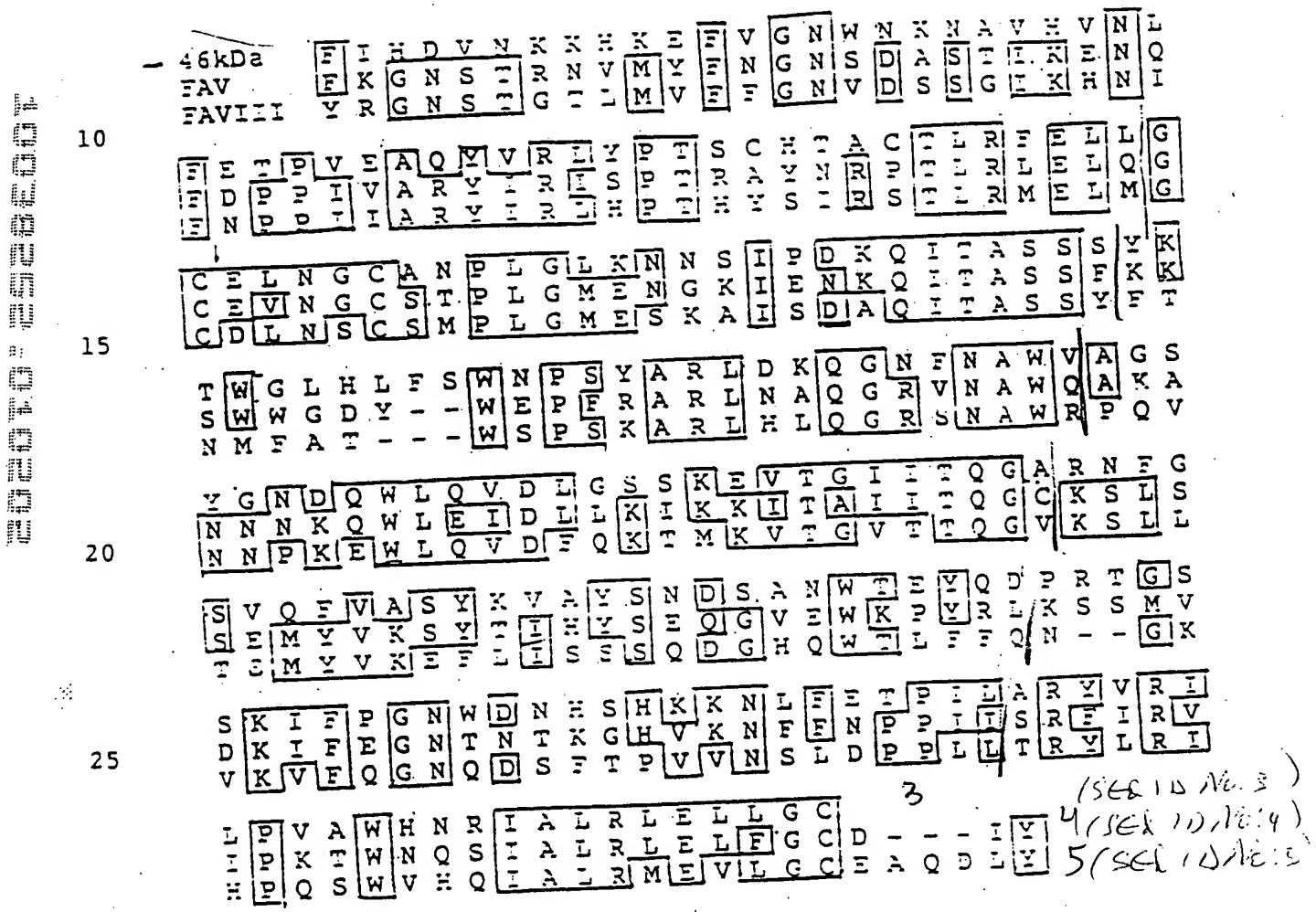
The partial sequence is 217 amino acids long and compounds to a theoretical molecular weight of about 24 kDa, representing the C-terminus of the complete protein. There are 4 potential sites for n-linked glycosylation. The sequence is asparagine and leucine rich.

Example 8: Homology to Clotting Factors

A comparison of the nucleotide sequence to the EMBL database using FSTNSCAN (PCGENE) revealed extended homology with human serum factors V and VIII and protein C.

The derived protein sequence, however, shares identity only with factors V and VIII, as shown in Table 2 below, but not with protein C, since the homology at the nucleotide level is in an intervening sequence.

Table 2: Comparison of Derived BA46-1 Amino Acid Sequence with C-terminal Human Serum Factors V and VIII.



An arrow indicates function of C1 and C2 repeats.

There is about 43% identity of BA46 to Factor V and about 38% to factor VIII. The region of factors V and VIII in Table 2 share about 47% identity.

Example 9: Study of Amino Acid Sequence

The analysis of the derived amino acid sequence of the about 46 kDa protein is consistent with its description as a glycosylated protein. The function of this protein, however, is unknown. Since the about 46 kDa protein has homology to both factors V and VIII, there may be a common ancestral protein to these serum clotting factors. The homology is in the C1, C2 region of the light chain of factor VIII (Arai, M., Scandella, D., and Hoyer, L.W. (1989) J.Clin.Invest., 83, 1978-1984).

Arai et al have shown that human antibodies that bind this region of the light chain, from hemophiliacs treated with factor VIII, inhibit factor VIII by preventing the interaction of factor VIII with phospholipids. Since this region has been implicated in phospholipid binding it is likely that it serves a similar role in the about 46 kDa glycoprotein.

The C-terminal portion may thus serve as a novel "anchor" sequence for the 46 kDa protein or it may possibly be involved in the binding of the mucin/membrane to the phospholipids on the surface of the growing milk fat droplet (Long, C.A., and Patton, S. (1978(J.Dairy Sci., 61, 1392-1399). Perhaps, it is involved in the assembly of the mucin complex at the plasma membrane surface.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.